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DUAL-RADIOASSAY TECHNIQUE: INDIUM-CHROMIUM MIXTURE.(U)  
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## DUAL-RADIOASSAY TECHNIQUE: INDIUM-CHROMIUM MIXTURE

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## NOTICES

This interim report was submitted by personnel of the Health Physics Branch and Radiation Physics Branch, Radiation Sciences Division, USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks Air Force Base, Texas, under job order SUPTXHPS.

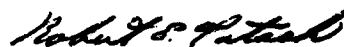
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This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.



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## DUAL-RADIOASSAY TECHNIQUE: INDIUM-CHROMIUM MIXTURE

### INTRODUCTION

A dual-radioisotope technique using In-111 and Cr-51 was recently used to investigate in vivo kinetics of total body distribution of two lymphocyte subpopulations (1). The time saved by this simultaneous determination was significant; however, assaying these two radioisotopes presented some unexpected problems due to the summing characteristics of In-111 photons. Indium-114m was considered as an alternate radioisotope because of its 192-keV photon emission and longer physical half-life (2). Here we present dosimetric data demonstrating the utility of In-114m in dual-radioassay studies.

Recently the labeling of isolated cells with In-111 has been reported as a new technique with important applications in clinical and research investigations. The introduction of indium as a radiolabel is significant because it is ideally suited for external monitoring of in vivo kinetics of total body distribution of cells. An impressive property of indium is that its lipid-soluble oxine complex incorporates into the cytoplasmic compartment of the target cell and binds to intracellular moieties, preventing rapid elution of the radiotracer in vivo. Platelets (3-9) and leukocytes from man and laboratory animals have been successfully radiolabeled and used in circulation studies. For example, in the clinical setting, transplanted In-111-labeled cells have been used for in vivo localization of pathophysiologic processes. In the research setting, however, the long-term fate of transplanted recirculating cells (e.g., homing of immunocompetent lymphocytes, involvement of platelets in the evolution of venous thrombi and damaged arterial intima, and localization of leukocytes in pathophysiologic processes at the site of an abscess or lesion) requires the use of a radiolabel with excellent photon emission and a relatively long half-life. Indium-114m possesses a long half-life and can be used to label cells of interest (1). Moreover, In-114m is energetically suitable for radioassay in the presence of Cr-51, a widely used radiolabel in long-term animal circulation studies. The present report demonstrates the efficacy of using indium and chromium in dual-isotope studies.

### MATERIAL AND METHODS

Radioisotopes were counted in a 1 3/4" x 2" (4.5 x 5 cm) NaI (Tl) well crystal. The spectra were stored in sections of a multichannel analyzer memory. Using the data-processing capabilities of the multichannel analyzer, the data were integrated over selected energy ranges to determine optimum window arrangements and cross-contamination; background was subtracted before processing each spectrum. After determination of the optimum windows and cross-contamination, dual radioassays were performed using a Nuclear Chicago auto gamma counter with a dual pulse-height analyzer.

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## RESULTS AND DISCUSSION

The physical characteristics of In-114m, In-111, and Cr-51 are shown in Table 1 (10). Indium-114m possesses a relatively long half-life ( $T_{1/2}$ , 50.1 days), compared to that for In-111 ( $T_{1/2}$ , 2.81 days). In addition, the In-114m radionuclide is essentially monoenergetic (192 keV), while In-111 exhibits two strong gamma emissions (172 and 247 keV) and a summation peak (421 keV). Chromium-51 is a relatively long-lived radionuclide (27.8 days) displaying monoenergetic gamma emission at 320 keV. These differences in photon emission are illustrated in Figure 1. The photon emission peaks from In-114m and Cr-51 are monoenergetic and significantly dissimilar: they display ideal emission properties for simultaneous radioassay of a mixed sample.

TABLE 1. PHYSICAL CHARACTERISTICS OF RADIONUCLIDES

Radionuclide	$T_{1/2}$ (days)	Type	Mean Energy (keV)	Mean Number of Disintegrations
In-114m	50.10	X-rays	192	0.1700
		Gamma 1	558	0.0350
		Gamma 2	724	0.0350
In-111	2.81	Gamma 1	172	0.8959
		Gamma 2	247	0.9395
		Sum Peak	~ 421	----
Cr-51	27.80	Gamma	320	0.1018

Simultaneous radioassay of mixed isotope samples was performed using a dual pulse-height analyzer operated with optimum window selections shown in Table 2. The cross-contamination shown at Cr-51 energies is significantly

TABLE 2. SUGGESTED WINDOW SELECTION FOR DUAL-ISOTOPE RADIOASSAY

Radionuclides	Window Width (keV)	Cross-Contamination
In-114m and Cr-51	150-240	8.2%
	280-380	15.9%
In-111 and Cr-51	130-270	14.6%
	280-350	18.3%

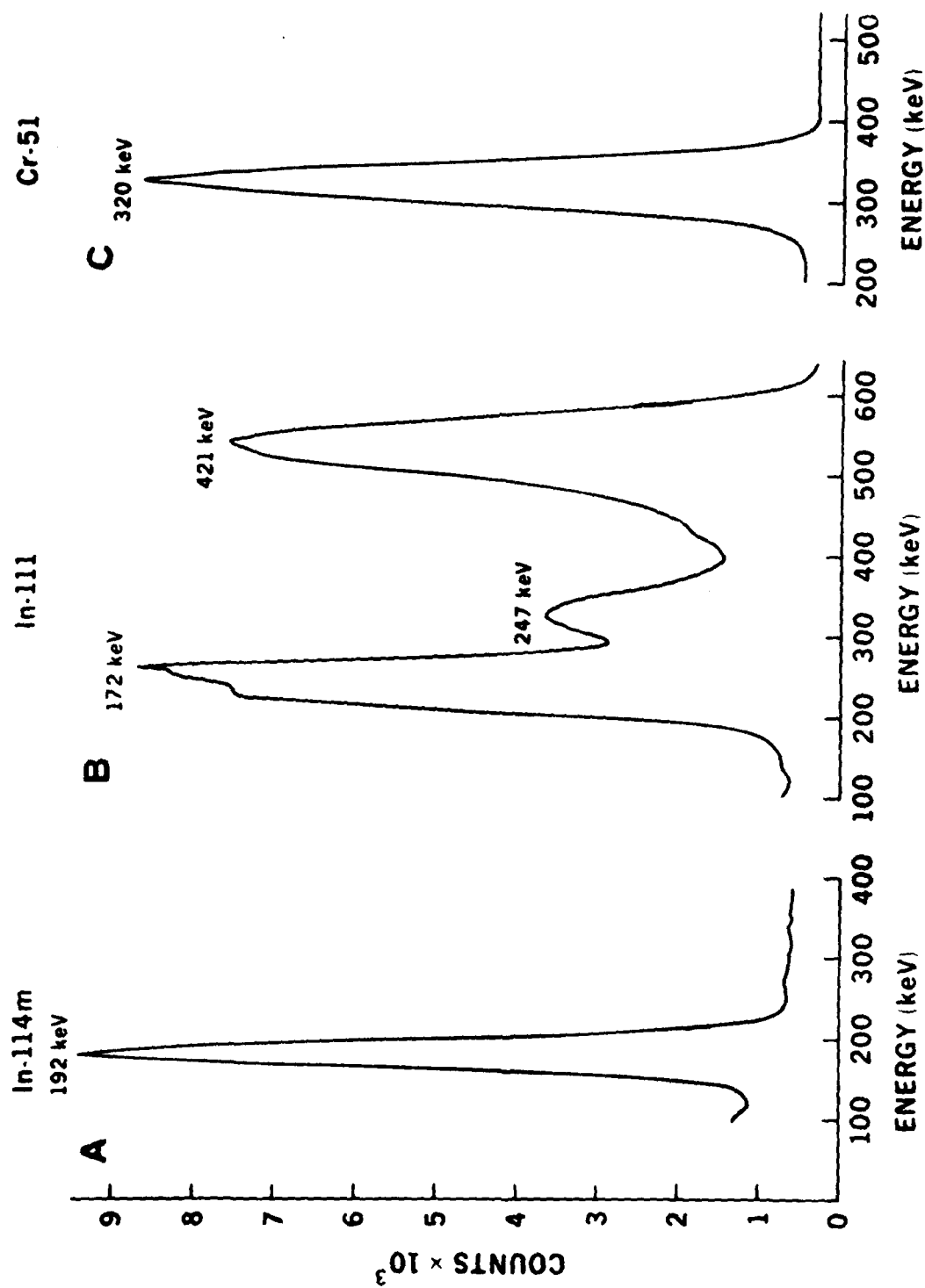


Figure 1. Energy spectra of radionuclides: Panel A. Indium 114m exhibits a prominent photopeak at 192 keV; Panel B. Indium-111 demonstrates a third sum-coincidence peak at about 421 keV; Panel C. Chromium-51 exhibits a monoenergetic gamma peak at 320 keV.

reduced for In-114m (8.2%) compared to that for In-111 (14.6%). To illustrate this difference, the energy spectra of In-114m and Cr-51 and of In-111 and Cr-51 are shown in Figures 2 and 3 respectively. Monoenergetic In-114m and monoenergetic Cr-51 exhibit representative photon emissions with little overlap (Fig. 2). In marked contrast, multienergetic In-111 and monoenergetic Cr-51 display a complex emission spectrum (Fig. 3) showing significant overlap of In-111 into Cr-51 energy levels. This overlap occurs because In-111 emits two gamma photons that undergo addition, creating the peak at 421 keV. This new 421-keV peak is identified as a sum-coincidence peak observed with certain multienergetic gamma and/or X-ray emitters (11).

The presence of a sum-coincidence peak complicates quantitative counting of In-111 since the magnitude of the sum-peak is characteristically sensitive to detector geometry. The effects of sample volume and source position on Indium-111 emission are shown in Table 3. Emission peak counts were made at 172, 247, and 421 keV for different window widths. Counting rate values were normalized to a value of 100% for the 1-ml sample volume and for a 0.0-mm sample elevation at the bottom of the well crystal. These data indicate that when the 421-keV sum-peak is included in the window, sensitivity decreases as sample volume increases, and as the source position increases within the well

TABLE 3. VOLUME AND SOURCE EFFECTS FOR IN-111

Peak (keV)	Case					
	A 172	B 247	C 172 247	D ~ 421	E 247 ~ 421	F 172 247 ~ 421
Window Width (keV)	150-224	226-322	150-322	327-480	226-480	150-480
Relative Counts						
Sample Volume (ml)						
1	100.0	100.0	100.0	100.0	100.0	100.0
2	101.0	99.6	99.9	97.6	98.5	101.0
3	99.9	99.7	99.8	95.2	96.9	99.9
4	99.6	100.9	99.7	91.5	94.3	98.3
5	96.1	99.9	96.8	85.8	90.3	95.1
Sample Elevation in Well (mm)*						
0	100.0	100.0	100.0	100.0	100.0	100.0
1	100.3	100.5	100.3	99.3	99.7	99.7
2	100.2	101.1	100.6	98.2	99.1	99.7
3	99.8	101.0	100.2	97.3	98.4	99.5
4	100.9	101.3	101.1	96.1	97.7	98.7
5	100.2	102.4	101.0	96.0	97.1	98.7

\*Sample Volume = 0.5 ml



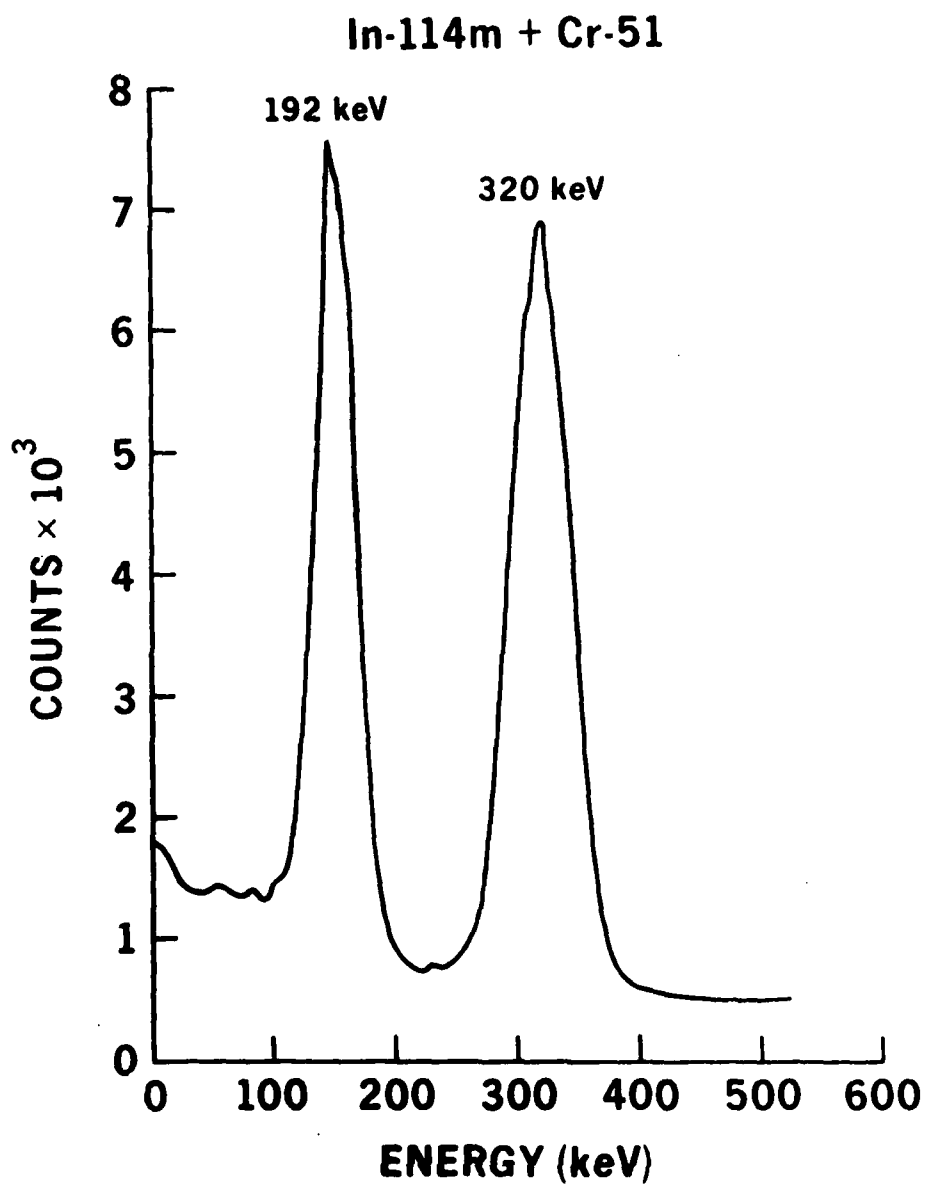


Figure 2. Energy spectrum of Indium-114m and Chromium-51. Peak separation is ideal for dual-label counting.

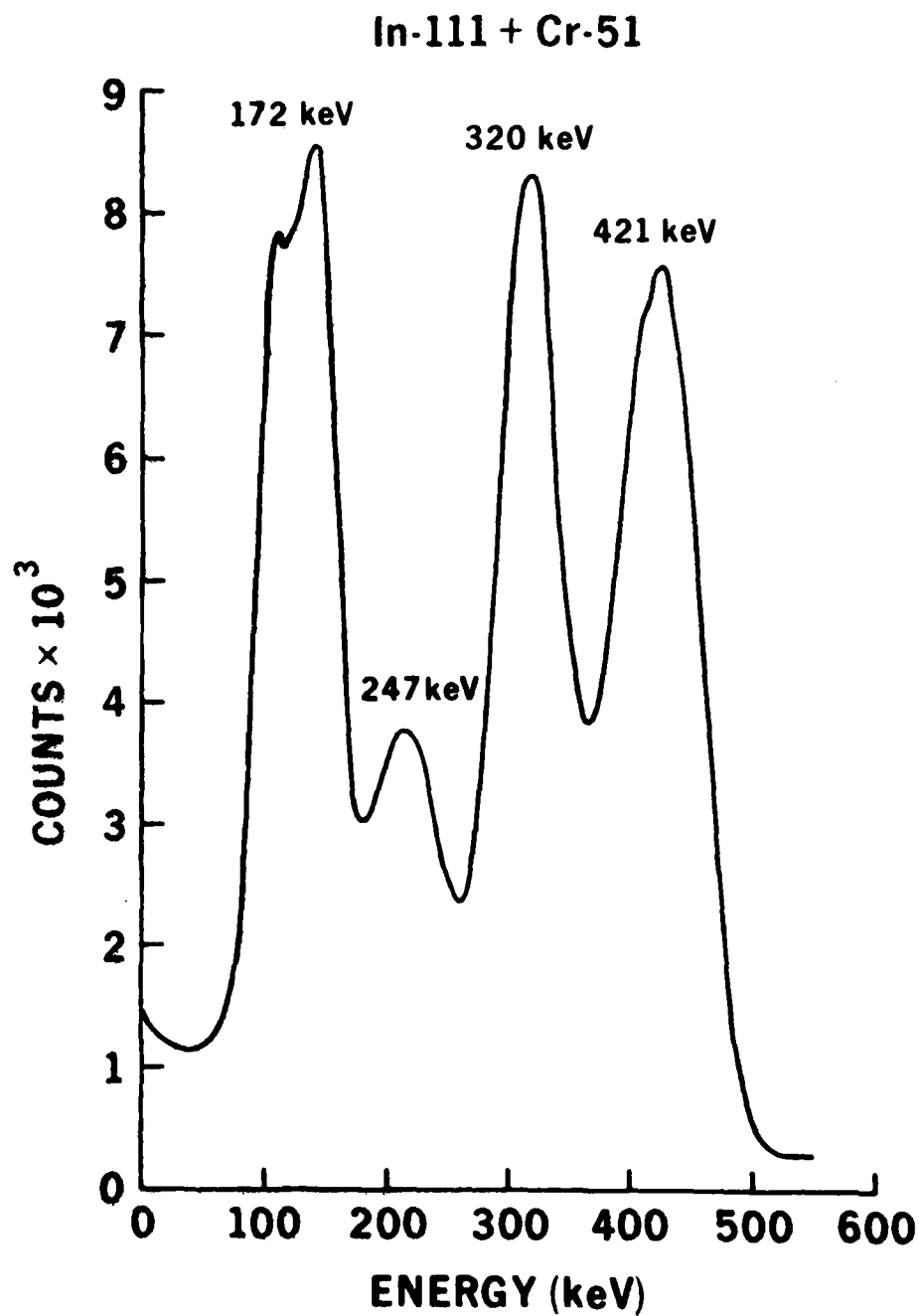


Figure 3. Energy spectrum of Indium-111 and Chromium-51 showing peak separation and sum-coincidence peak. Peak separation is not ideal for dual-label counting.

crystal (Cases D, E, and F). This is because each gamma photon can leave in any direction, and as crystal-to-source geometry improves, the probability of the two photons interacting simultaneously in the crystal and being summed together is greatly enhanced. Volume and sample geometry effects become less pronounced when the 172-keV and the 247-keV peaks are counted together (Case C) or when each is counted alone (Cases A and B). The suggested window settings for In-111 and Cr-51 when counted as a mixed sample are summarized in Table 2. These selected window arrangements are typical for our instrument and should be used only as a guideline for other instruments.

### CONCLUSIONS

Data presented here indicate that the dual-radioassay technique can be applied to indium-chromium mixtures. The physical characteristics of In-114m, its geometry independence, and the ease of separating its photopeak from chromium make it ideally suited for simultaneous radioassay applications. These properties also make In-114m an isotope of choice in cell-labeling research designed to study the long-term fate of cell recirculation. For example, one particularly important application is in immunologic investigations to study the in vivo interactions of functionally distinct lymphocyte subpopulations (1). In all applications, however, a properly calibrated pulse-height analyzer is essential for quantitative dual-radioisotope counting. We recommend that spectra of indium and chromium alone and of indium and chromium mixed be determined in order to optimize window settings for each gamma counter.

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